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Distribution and Source of Polycyclic Aromatic Hydrocarbons (PAHs) in the Aquatic Species of Iraqi Marine Waters

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Abstract: ***This work was carried out to assess the levels of Polycyclic Aromatic Hydrocarbon (PAHs) in selected fish species. Concentrations of PAHs were determined in edible tissues of (Nemipterus japonicas, Cynoglossus arel and pomadasys maculatus). PAHs levels in the samples were measured by gas chromatography with flame ionization detector (GC/FID). The present article, the distribution and sources of 16 polycyclic aromatic hydrocarbons (PAHs) in three fish species were collected from Iraqi marine waters during 2016 were investigated. The concentrations of ∑PAHs ranged from 21.879 to 141.561 ng/g (dry weight) The concentrations of PAHs in this study were higher in the winter season. The results showed that the contamination is more likely occurred as a result of their ingesting contaminated material than direct body exposure to oil residues in the water column. The ratios of The LMW/HMW anthracene/(anthracene + phenanthrene) and fluoranthene/ (fluoranthene + pyrene) ratios indicated that the source of PAHs compounds was mainly pyrogenic and petrogenic .reflected a pattern of both pyrolytic and petrogenic input of PAHs in. PAHs ranged from a relatively moderate to high PAHs pollution compared to other urbanized coastal areas worldwide. The studied area received their PAHs from both petrogenic and pyrogenic sources, although petroleum derived PAHs were dominant at the stations situated near the refinery processes and pyrolytic sources were mainly prevalent in the drainage***

Keywords: polycyclic aromatic hydrocarbons (PAHs); Iraqi marine waters

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group complex mixture with more than 10,000 individual compounds formed by two or more fused aromatic rings of carbon and hydrogen atoms originating from natural and anthropogenic sources, such as incomplete combustions, industrial incinerations, transport or uncontrolled spills (Nácher-Mestre *et* al., 2010).

In recent times, PAHs have received much attention due to their potential adverse human health and ecosystem impacts (Nkpaa,*et* al.2013). Human exposure to these pollutants can result in cancer, mutations and birth defects. Adverse effects of PAHs have also been observed in marine organisms and they include growth reduction and DNA damage Ingestion of contaminated food and diffusion from water across their gills and skin are the major routes of PAHs exposure to fish. Due to the lipophilic nature and high chemical stability of PAHs (Caliani *et. al.*, 2009). they accumulate in the fatty tissues of fish following their uptake Fishes are, therefore, good indicators of pollution in coastal waters, and they have been used extensively for environmental monitoring(Nyarko, *et. al*., 2011).

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous anthropogenic pollutants that can be biologically amplified to high concentrations in food webs. Due to their lipophilicity, persistence, and high toxicity, these residues are readily accumulated in the tissues of non-target living organisms where they may cause detrimental effects. PAHs are toxic, carcinogenic, and mutagenic to all organisms, including humans (Nacci,*et* al.,2002; Armstrong,*et* al. 2004; Bolden, *et* al. 2017).

Pollution by persistent chemicals is potentially harmful to the organisms at higher tropic levels in the food chain. Fishes are, therefore, good indicators of pollution in coastal waters, and they have been used extensively for environmental monitoring**,** fish is a major source of proteins and healthy lipids for people so the marine organisms that like fish are able to accumulate several fold higher concentration of PAHs than the surrounding water, **(**Nyarko, *et* al., 2011**).**

The sources of PAHs in the coastal environment are described as either petrogenic (if the source is derived from petroleum, e.g. natural oil seepage and oil spills) or pyrogenic (if the source is derived from the incomplete combustion of organic matter and fossil fuel (Baumard *et. al*., 1998; and Abrajano *et. al*., 2003). Low molecular weight(LMW) PAHs, containing two or three fused rings, are more water soluble and volatile, therefore they are more available, than high molecular weight (HMW) PAHs,containing four or more fused rings , hence (LMW)PAHs are more susceptible to various degradation processes (Bakhtiari *et* al., 2009).

The ratio of high molecular weight PAHs (HMW-PAHs) to low molecular weight PAHs (LMW- PAHs) has been used to characterize the origin of PAHs in the environment (Nyarko, *et. al*., 2011). It is reported that the oil contamination in Shatt Al-Arab River was possibly originated from diverse sources including oil refineries, industrial waste, rural runoff, electricity generating stations, sewage discharges and river transportation activities (Farid *et al*., 2014).

In Iraq there are many studies on the presence of petroleum hydrocarbons in the coastal water and fish (Al-Saad , *et* al., 2016a;Al-Ali,*et* al. ,2016 ;Al-Khion,2012 ; Al-Khion,*et* al.,2016) freshwater and fish(Ali , *et* al.,2013; Al-Saad ,*et* al.,2016b) ., soil and sediment (Farid *et* al., 2016). However, there is no information available concerning dietary intake of PAHs and their risk from fish consumption.

The objectives of the study were therefore to determine the concentrations of polycyclic aromatic hydrocarbon (PAHs) in three commercially important fish species from marine waters, and to identify the major sources of the polycyclic aromatic hydrocarbon (PAHs) to give baseline data for further work.

2. Material and Method

Study area

Marine Iraqi coastal has a tragic history of pollution from oil spills and oil well fires; although no systematic scientific information has been available about the ensuing contamination. Is a region covering 836.83 $Km^2$and include the mudflats confrontation the coastal area 146 Km^2, and half the size of Khor Abdula until Al-Damah 162(3,146 Km^2) and half the size of Khor Shitanh (1,36 Km^2) in addition Khor Al-Zoubair (Ali and Ahmed ,2015).

Sample Collection. Three species of fish samples were collected during winter and summer of 2016. Species which were commonly available, namely *N. japonicas, C. arel* and *P. maculatus* in the study locations were collected with the help of local fisherman of the region, and the morphometric on collection, fish samples were stored in pollution-free polythene covers and transported to the laboratory, in ice box and stored at −20◦C in the deep freezer until analysis.

Sample Processing: Fish samples were taken out from the deep freezer, reweighed and stored in a desiccator. Prior to extraction, the freeze-dried samples were ground to powder using pestle and mortar to ensure homogenization. Were dried by a rotary vacuum evaporator to remove the lipids from the solvent. The percentage of lipid was estimated by calculating the differences of weight using a sensitive balance. The powdered samples were then Soxhlet extracted according to Grimalt and Oliver (1993). The extract was reduced to drought and the remainder was melting with hexane for measuring the total concentrations of aromatic hydrocarbon compounds using A Schimadzu RF-540spectrophotometer device. The emission intensity was measured at 360 nm with excitation set at 310 nm.

The extracts were then fractionated into aliphatic and aromatic hydrocarbons by chromatography column. The column was prepared by slurry packing 10 g of silica (100-200 mesh), followed by 10 g of alumina (100-200 mesh) (silica-gel and alumina were activated at 200 ºC for 4 hours and then partially deactivated with 5 % water) and finally 1 g of anhydrous sodium sulphate was added to the surface to avoid disturbance of the top layer when pouring the solvent. The extract was then applied to the head of the column and eluted with 20 ml n-hexane to obtain on the aliphatic fraction and 20 ml of benzene to yield the aromatic hydrocarbons. The aliphatic fraction saved for further analysis and the aromatic fraction were only concentrated on a rotary evaporator, transferred to a vial, and the volume was adjusted to 1 ml exactly using a stream of N2. An aliquot of 1 µl of extract of aromatic hydrocarbons was subjected to analysis by a Allegiant capillary gas chromatography with flam ionization detector (FID). The fused silica capillary column (100 m x 250 µm x 0. 5 µm) used was a wall coated open tubular (methyl silicone) (Agilent US2463233H DB-petrp), with helium as gas carrier at flow rate of 1.5 ml/minute. The operating temperatures for detector and injector were 300 °C and 320 °C, respectively. The temperature of column was held at 50 °C for 8 minute then 8 °C /minute to 350°C. The individual PAHs were identified based on the retention time of an authentic PAHs mixed standard procured from Supelco, USA. The concentrations of PAHs compounds were calculated based on the standard calibration curve of corresponding standard compounds. Recovery assays for standards aromatic compounds ranged from 80 % to 92 %. Standard deviation for the method was less than 10 % based on replicate analysis. Great care was taken to avoid contamination of the samples throughout the analytical procedure. All solvents were distilled twice before use; glassware was rinsed with distilled water and heated in an oven at 450 °C for 24 hours. However, procedural blanks consisting of all reagents and glassware used during the analysis were periodically determined which had shown no detectable interference.

Information regarding sampled species, lipid content, length, and weight is presented in Table 1.

Table 1: Details information of the collected fish samples

	Winter			Summer		
Fish species	% lipid content	Mean length	Mean width	% lipid content	Mean length	Mean width
N.japonicus	0.2922	15.3	17.06	0.0017	10.05	8.05
P.maculatus	0.2956	17.7	7.8	0.2426	7.6	5.5
C. aeel	0.3426	17.6	32	0.2741	8.6	14.32

Some of ratios used to determine sources of aromatic compounds are represented as including the following : (Fl/Py), (Phen/Ant), (LMW /HMW), (Ant / (Ant + Phe)) and (Bap / (Bap + Chr)). The calculation of these concentrations can be an indicator of the source of the aromatic hydrocarbons found in the fish samples, ratio of (Fl/Py) Values greater than 1 have been used to indicate pyrogenic origins and values less than 1 are attributed to petrogenic source (Qiu *et al.*, 2009) . Ratio of (Phen/Ant) less than 1 indicates petrogenic (Zhu *et al.*,2004) . ratio of(LMW /HMW) values greater than 1 indicate petrogenic origins and values less than 1 are attributed to pyrogenic sources (Vrana *et al.*, 2001).

(Ant/(Ant+Phe)) ratio presumes that ratios less than 0.1 indicate PAHs source to be of petroleum origin while ratios larger than 0.1 indicate PAHs source to be of combustion origin and the ratio of (BaA /(BaA+CHR)) less than 0.2 implies petrogenic, from 0.2 to 0.35 indicates either petrogenic or pyrogenic origins , and larger than 0.35 implies pyrogenic sources (Guo et *al.*,2007) .
(Fl/Py) = fluoranthene to pyrene.
(Phen/Ant)= Phenanthrene to Anthracene .
LMW (low molecular weight) = Naphthalene + Acenaphthylene + Acenaphthene + Fluorene+ Phenanthrene +Anthracene.

HMW(high molecular weight) = Fluorananthene + Pyrene + B a A+ Chrysene + Bbf+ BK f + BaP + DBA + B (ghi) perylene +indenol pyrene.

(Ant / (Ant + Phe)) = Anthracene to Anthracene + Phenanthrene .

(Bap / (Bap + Chr)) = benzo(a) anthracene / (benzo(a) anthracene + chrysene).

3. Result and Discussion

All samples were analyzed for 15 USEPA priority PAHs, including naphthalene (NaP), acenaphthene(Ace), acenaphthylene (Any), fluorine (Fl), phenanthrene (Phe), anthracene(An), fluoranthene (Fla), pyrene (Pyr), benz[a]anthracene (BaA), chrysene(Chr), benzo [b]fluoranthene (BbF), benzo [K]fluoranthene (BKF), benzo[a] pyrene (BaP), dibenz[a,h]anthracene (dBA), and benzo[g,h,i]perylene (BPe), also measured and quantified according to the respective peak areas.

A total of 15 PAHs were analyzed in *N .japonicus* , *P.maculatus* and *C. aeel* from Iraqi Marine water . The total of these PAHs in winter vary from 141.56-188.98(ng g^{-1} dry weight) and in summer ranged from 21.87-80.31 (ng g^{-1} dry weight). The concentration of PAHs in *N. japonicus* ranged between 5.8012 to 12.6117(ng g^{-1} dry weight) in Winter and in Summer ranged from 0.7062 to 4.2072 (ng .g^{-1} dry weight) ,while in *P.maculatus* ranged from 3.6199 to 59.9296 (ng. g^{-1} dry weight) in Winter and ranged from 0.8473 to 28.5196 (ng .g^{-1} dry weight) in Summer, but in *C. aeel* concentration ranged from 3.3928 to 62.7389(ng .g^{-1} dry weight) in Winter and in Summer from 0.9207 to 22.8961 (ng .g^{-1} dry weight) (table 2,3). In this study levels of PAHs was higher than as reported in Al-Khion *et* al.(2016) ranged between (0.432) to (14.939) ng/g dry weight . Due to increased oil activity in the Shatt al-Arab and Marine water through the loading and unloading of ships and through the tides (AL-Saad, *et* al. ,2017).

Table 2: Concentrations of PAHs (ng .g^{-1} dry weigt) and isomeric compounds of pyrogenic and petrogenic origins in the fish samples from Iraqi marine water in Winter

Name compound	*N. japonicus*	*P.maculatus*	*C. aeel*
Naphthalene	**5.80**	**11.04**	**8.71**
Acenaphthylene	**9.98**	**9.91**	**9.61**
Acenaphthene	**9.55**	**9.47**	**9.73**
Fluorene	**11.17**	**10.19**	**9.95**
Phenanthrene	**10.22**	**9.81**	**9.80**
Anthracene	**11.27**	**10.93**	**10.74**
Fluoranthene	**10.53**	**9.62**	**9.69**
Pyrene	**10.24**	**9.56**	**9.84**
Carbazol	**10.25**	**-**	**9.69**
Benzo[a]anthracene	**12.61**	**59.93**	**62.74**
Chrysene	**-**	**24.75**	**25.21**
Benzo[k]fluoranthene	**10.08**	**3.62**	**3.39**
Benzo[a]pyrene	**9.90**	**9.81**	**9.87**
Dibenzo[a,h]anthracene	**9.97**	**-**	**-**
Benzo[g,h,I,]perylene	**9.99**	**8.89**	**-**
∑ PAHs	**141.56**	**187.53**	**188.99**
fluoranthen/pyrene	**1.03** pyrogenic	**1.01** pyrogenic	**0.99** petrogenic
Phen/Ant	**0.91** petrogenic	**0.90** petrogenic	**0.913** petrogenic
Ant/(Ant+Phen)	**0.52** pyrogenic	**0.53** pyrogenic	**0.52** pyrogenic
BaA/(BaA+Chry)	**1** pyrogenic	**0.28** petrogenic+ pyrogenic	**0.28** petrogenic+ pyrogenic
LMW /HMW	**0.69** pyrogenic	**0.49** pyrogenic	**0.45** pyrogenic

Table 3: Concentrations of PAHs (ng g^{1}dry weight) and isomeric compounds of pyrogenic and petrogenic origins in the fish samples from Iraqi marine water in Summer

Name compound	*N. japonicus*	*P. maculatus*	*C.aeel*
Naphthalene	**1.61**	**28.52**	**22.90**
Acenaphthylene	**1.04**	**3.82**	**1.98**
Acenaphthene	**3.69**	**1.76**	**3.24**
Fluorene	**4.21**	**2.17**	**4.57**
Phenanthrene	**0.71**	**3.92**	**1.38**
Anthracene	**1.17**	**3.22**	**1.88**
Fluoranthene	**1.27**	**4.49**	**2.84**
Pyrene	**3.79**	**2.51**	**4.76**
Carbazol	**2.17**	**0.85**	**0.92**
Benzo[a]anthracene	**1.28**	**20.92**	**13.41**
Chrysene	**0.96**	**5.24**	**2.51**
Benzo[k]fluoranthene	-	-	-
Benzo[a]pyrene	-	**1.85**	-
Dibenzo[a,h]anthracene	-	**1.07**	-
Benzo[g,h,I,]perylene	-	-	-
∑ PAHs	**21.88**	**80.32**	**60.38**
fluoranthen/pyrene	**0.33** petrogenic	**1.79** pyrogenic	**0.60** petrogenic
Phen/Ant	**0.61** petrogenic	**1.22** pyrogenic	**0.74** petrogenic
Ant/(Ant+Phen)	0.62 pyrogenic	0.45 pyrogenic	0.58 pyrogenic
BaA/(BaA+Chry)	-	**0.26** petrogenic+ pyrogenic	-
LMW /HMW	**1.31** petrogenic	**1.18** petrogenic	**1.47** petrogenic

The highest concentration recorded was obtained in winter , while the lowest concentration recorded in summer fig(1). This may be related with the climatic condition of Iraq and the effect of photo oxidation, volatilization and high degradation during the hot season (Al-Saad *et* al. ,2016). The highest concentrations were recorded at due to hydrophobicity nature of PAHs and their affinity to organic material (Zakaria et al., 2002; Maskaoui et al., 2002).

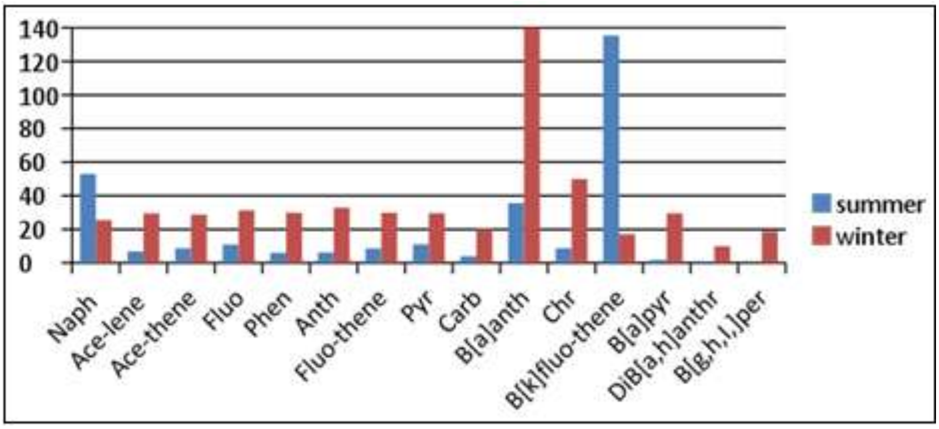


Figure 1: The spatial profiles of the total concentrations of PAHs in samples

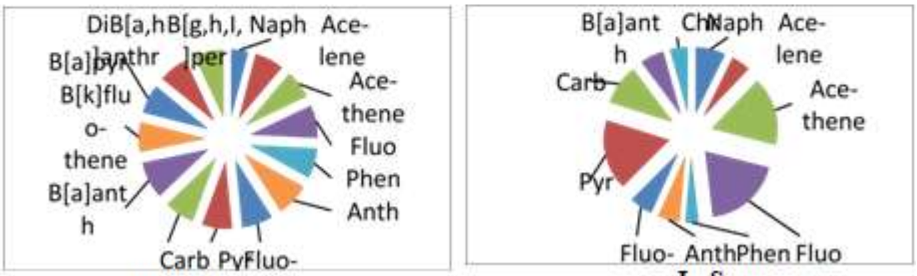
Some ratios were calculated as attempt to identification the sources of the aromatic compounds, in the current study indicates that the source of PAHs compounds varied between petrogenic and pyrolytic origin (Tables 1,2) .

The various components of PAHs in present studies Flut, Pyr and Ant observed in *N. japonicas* (Figure 2) and the presence of most other components at lower rates in the two essences . Residual levels of Nap, Chy and BaA were high in the tissues of *C.arel* and lake of BkF (Figure 3).

It is obvious from (Figure 4), that Nap, Chyr and BaA are abundant in *p. maculatus* and no presence of observed DahA and B(g,h,i)per .

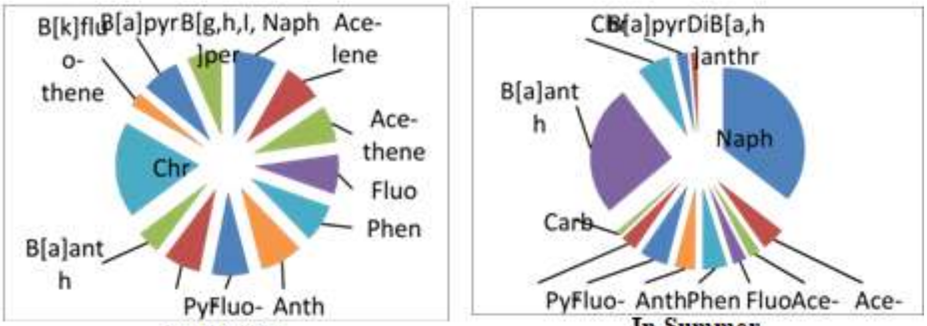
Results of the study showed Fluorene was the most frequently detected as reported in other studies (Hamad,*et al.* 2014;Liang, *et al.* 2007) , followed by Benzo[a]anthracene and Acenaphthene . Indeno[1,2,3-cd] pyrene could not be detected in any fishes accept in *Ch.dorab* ,while the absence or rather low detection of certain PAHs in the fish samples may be attributed to their rapid depuration or biotransformation (Deb, *et al.* 2000; Thompson ,*et* al.2017). The accumulation and depuration of PAHs in fish can be influenced by various factors including route and duration of exposure, lipid content of tissues, environmental factors, differences in species, age, and sex, and exposure to other xenobiotics (Varanasi, *et al.* 1987).

Chrysene is a preserved biomarker PAH and was chosen to be a good marker of petroleum compounds because of its resistance to abiotic factors and the degradation of the microorganisms (Yang, 2000). The PAHs compounds are of concern because of their acute toxicity, mutagenicity, or carcinogenicity (Wang *et al*., 2011).



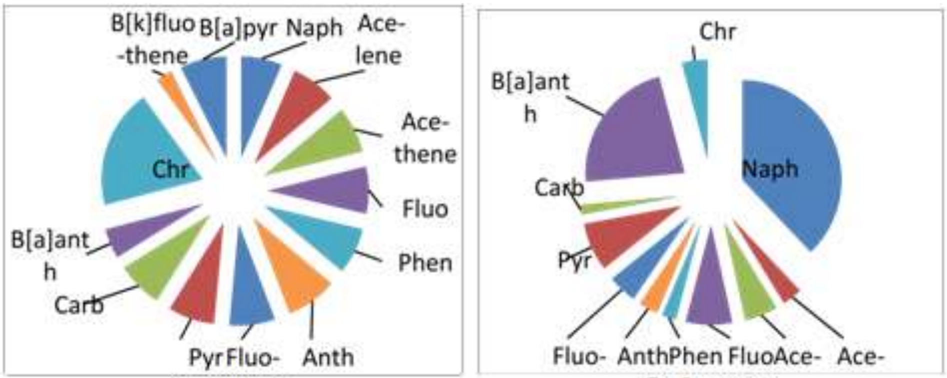
In Winter **In Summer**

Figure 2: The percentage of the PAHs compounds in *N. japonicas*



In Winter **In Summer**

Figure 3: The percentage of the PAHs compounds in *C.arel*



In Winter **In Summer**

Figure 4: The percentage of the PAHs compounds in *p.maculatus*

By calculating the value of LMW/HMW ratio in Summer value were >1 , and this is indicate the main source of the PAHs in the investigated area coming from petrogenic source.

The less carcinogenic polyaromatic hydrocarbon of lower molecular weight (LMW PAHs) observed naphthalene was detected clearly in all the studied fish species (Table 2,3). The high molecular weight compounds that more carcinogenic (HMW) PAHs (BaA, BbF, BkF, BaP, and InP) were low detected in the fish samples analyzed.

This shows that the source of PAHs in the river was mainly from petrogenic sources. The ratio of low molecular weight PAHs (LMW- PAHs) to high molecular weight PAHs (HMW-PAHs) has been used to characterize the origin of PAHs in the environment (Olaji, *et* al. 2014). The accidental discharge hazardous materials such as petroleum and chemical solvents to the aquatic environment has become the focus of increasing regulatory and public concern because of the adverse impacts of such materials on human health and the environment (Al-Shwafi,2008) .

The total PAHs concentration reported in fish samples of the present study appears to be higher than the concentration reported in edible fishes (0.207–3.365 ppm) of the Gomti river, Lucknow, India (Malik,*et al.* 2008). They are comparable with that recorded for the Saudi Arabia coasts; 1.88-412 ng/l in Arabian Gulf (Awad , 1990) .

By comparing the present data with those reported by FAO/WHO for the guidelines of PAHs in food with value (0.001) for human consumer, it's clear that the present data it can be noticed that the levels of PAHs compounds in the muscles of these fishes were approach to their levels in other fishes collected from other regions of Iraq and the world (Table 4).

Table 4: Comparison of PAHs levels in fishes samples with those in other studies in Iraq and the world.

References	Concentrations (ng.g^{-1}) d.w	location
DouAbul,*et al.*1997	14-106	North West of Arabian Gulf
Fowler, *et al.*1993	7.6-239	Saudi Arabia
Fowler, *et al.*1993	2.2-110	Oman
Fowler, *et al.*1993	55.8-165.9	Qatar
Sreenivasa, and RAO 2001.	0.059- 0.183	India
Al-Saad,*et al.*2006	23.90-57.90	Red sea coast of Yemen
Al-Saad,*et al.*2006	6.78-23.83	North West of Arabian Gulf
Al-Khion ,2012	1.83-84.67	Iraqi coast regions
Hamad,*et al.*2014	2.4093 - 5.5102	Libya
Olaji,*et al.*2014	0.003- 0.038	Nigeria
Abdolahpur Monikh,*et al.*2014	91.32 - 11 54.45	Arabian Gulf
Jazza,*et al.*2015	1.095 -16.661	Al-Kahlaa River – Missan-Iraq
Al-Khion ,*et* al.2016	0.432-14.939	Iraqi marine water
Nascimento,*et* al.,2017	11.45 - 1825.35	São Paulo River estuary- Barazil
Present study	21-188	Iraqi marine water

The PAHs concentration detected in the fishes of present study is comparable with study reported in the muscles of the fish, from the Derna city coast (1.51 – 8.23 µ g/g dry wt.) (Hamad,*et al.* 2014) , and lower than the levels in the Red Sea Coast (57.90 ng/g dry wt.)(Al-Saad , *et al.* 2006) .

Polycyclic aromatic hydrocarbons (PAHs) are a class of common persistent environmental pollutants found in water, air, soil, and plants and can be released by natural sources. However, the majority of atmospheric PAHs are from vehicular emissions, coal-burning plants, and the production and use of petroleum-derived substances. Exposure to PAHs has been implicated in cancer and other diseases. (Bolden,*et* al. 2017).

When comparing the results with the Baumard scale we find that they range from low to moderate (Baumard,*et* al. 1998). Further studies should be performed to investigate the effects of PAHs in the Shatt Al-Arab River in order to provide minimum acceptable levels of this compounds within this area to humans, fauna, and flora.(Farid,*et* al.2016). Using fish muscle is very important to determine the levels of hydrocarbons because the analysis of fish's muscles would give good information about petroleum hydrocarbon(Hantoush *et* al. ,2001).

As a conclusion, these fishes are contaminated with PAHs,while these fishes are commercial and eaten by people .The proportions of PAHs were they are higher than in the Arabian Gulf, so that they are not recommended to increase of eating them and the main sources of these compounds petrogenic or pyrogenic origins.

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